Vaccines have proven to be one of the most successful and efficient means of controlling infectious diseases of public health importance, including smallpox, polio, pertussis, and measles (1). The annual global burden of 300–500 million new malaria cases and 1–3 million malaria-related deaths demonstrates the potential public health and socioeconomic benefit that would accrue with the deployment of a highly effective malaria vaccine, particularly for the most affected and impoverished populations (2). However, despite several decades of research and substantial evidence for its feasibility, such a vaccine remains elusive (3).

Among the many challenges facing vaccine developers is how to mine the human immune response to natural infection to identify protective antigens and associated host responses. While there is no evidence that sterile immunity is ever naturally induced by the parasite, both symptoms and parasite density gradually decline with repeated exposure, resulting in a state of clinical and partial parasitological immunity (4). Thus young children living in endemic areas are at the greatest risk for high-density infections and for clinical disease, including severe complications, whereas older children and adults are rarely symptomatic, although they may harbor and transmit the parasite. A variety of data implicate antibody responses to multiple blood stage antigens as the primary effector mechanism underlying naturally acquired immunity; the antigenic targets, however, have not been clearly identified. Our limited understanding of the array of protective (and nonprotective) immune responses is reflected by the small number of antigens under development as candidate vaccines or shown to be protective in experimental challenge models (5, 6). Indeed, current generation subunit vaccines are based on a few “traditional” antigens (7) despite evidence that antimalarial immunity is likely multifactorial, targeting many antigens and involving antibody responses, T cell responses, cytokines, natural killer cells, and many additional effector mechanisms (8, 9, 10).

A complete understanding of the array of malarial antigens contributing to clinical immunity and the associated immune responses would be immensely beneficial to vaccine developers but has not been achieved, due in part to the limitations of available immunologic techniques. For example, traditional immunoassays such as ELISA, while useful in assessing specific immune responses, allow for the analysis of just one antigen at a time and give little indication of antibody functionality. Performing these assays is time-consuming and requires a large number of samples, and therefore has been limited in scope. To adequately screen immune responses from multiple individuals against a large number of antigens has not been feasible, frustrating efforts to identify broad patterns of protective responses against malarial antigens as well as individual differences in the ability to respond successfully to multiple targets.

Recent advances in the fields of genomics, proteomics, and molecular immunology are creating opportunities to conduct unprecedented in-depth investigations of the parasite genome and the host immune response, and provide promise of overcoming these limitations. In this issue of Clinical Chemistry, Gray et al. (11) report the results of a study using microarray technology to characterize serum reactivity profiles of 189 children, 3–9 years old, in The Gambia who have been grouped according to level of clinical immunity to Plasmodium falciparum malaria. In this investigation, the authors simultaneously assessed the antibody responses against 18 recombinant proteins derived from 4 leading blood stage P. falciparum vaccine candidates (the majority of the proteins derived from MSP1) during one malaria transmission season. The authors found an unexpected complexity of antibody responses, in that the microarray analysis revealed 189 distinct reactivity profiles, with each child recognizing different combinations of antigens. Using k-means clustering and phylogenetic networking as 2 independent analytical techniques, the authors were able to demonstrate that certain combinations of antigen-antibody activity, rather than reactivities to any single antigen, were associated with clinical protection. For example, reactivity profiles in cluster 1 generally recognized AMA-1 and the 2 variants of MSP2 (FC27 and 3D7), and this cluster contained a statistically significantly higher number of children from the clinically immune group relative to the other clinically defined groups. In contrast, no significant associations between clinical protection and responses to any single antigen were identified. These results add substantively to our understanding of protection against malaria by illustrating that protection may be a higher-order phenomenon related to patterns of response and not attributable to any single antigenic target. In addition, the study revealed the potential limitations of previous work demonstrating correlations between protective immunity and immune recognition of single antigens.

Gray et al. (11) reported on proteins derived from only 4 merozoite antigens, and therefore did not fully explore the higher-order patterns that might have been revealed had a larger number of blood stage antigens been tested. Antibody responses to preerythrocytic stages of the parasite would also be of interest, now that it has been demonstrated that immunization with a preerythrocytic-stage antigen can lead to a reduction in clinical episodes of malaria (12), although in the case of preerythrocytic-stage antigens, T cell responses rather than antibodies may be the primary effector mechanism underlying protection.

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The ability of microarray assays to detect specific antibody responses to multiple antigens in parallel has significant clinical and research implications. This methodology has already been shown to be useful in the serodiagnosis of infectious diseases, such as toxoplasmosis, rubella, and herpes simplex viruses (13), and may soon replace ELISAs in clinical laboratory settings. Microarray analysis to obtain antibody reactivity profiles has also been shown to be useful in vaccine development against other organisms. Li et al. (14) used protein microarrays to profile antibody responses of immunized rabbits against 149 Yersinia pestis proteins, and identified 11 novel proteins that will be further studied as potential candidates for subunit vaccines. Similarly, antibody response profiles of women infected with Chlamydia trachomatis were assessed against 156 chlamydial fusion proteins using protein microarrays. Although many of the 15 women demonstrated distinct reactivity patterns, certain fusion proteins were commonly recognized and led to the discovery of 5 novel immunoreactive antigens (15). Now, the investigation by Gray et al. (11) demonstrates that this approach can be used to characterize reactivity profiles against malarial antigens.

The question arises as to whether microarray analysis can prove as useful to malaria vaccine development as it has to the development of vaccines to other organisms. Compared to most bacteria and viruses, the Plasmodium parasite is much more complex (>5300 genes) and elicits a greater variety of specific immune responses. Using microarray technology to obtain serum reactivity profiles against different malarial antigens may result in an overwhelming amount of data that is difficult to effectively analyze and interpret. Recently, issues of reproducibility and threats to the validity of investigations using serum proteomics have arisen. Studies in the field of oncology have used protein microarrays to assess serum profiles for the identification of biomarkers of cancer outcomes, and several years ago a study reported almost 100% sensitivity and specificity of a test using pattern-recognition proteomics analysis of serum for detection of ovarian cancer. Plans to market this test were initiated and then halted owing to issues of reproducibility and reliability of the technology (16). Proteomic patterns are difficult to reproduce from one dataset to another, and the statistical methods to analyze data may not classify the data accurately, particularly across datasets or experiments (17). Furthermore, in non–hypothesis-driven discovery-based investigations, overfitting the data can occur. Often microarray analysis is conducted using a multivariable model designed to discover a “pattern” that discriminates among individuals in one group vs another (e.g., clinically immune vs nonimmune). When assessing a large number of possible responses, a perfectly fitting pattern can occur by chance alone, overfitting the data, which subsequently has “no discriminatory ability that can be reproduced in individuals different from those used to derive the model” (18). Because the serum responses to malarial antigens can be very diverse and the antigens numerous, investigators using this technology must be aware of these issues and use appropriate design and statistical methods to avoid threats to validity and ensure the reproducibility of results. Although these cautionary notes are aimed at future users of this approach and not at the work by Gray et al. (11), it would be extremely interesting to see if the patterns identified in this seminal paper can predict clinical status during an additional period of surveillance for symptomatic parasitemia in The Gambia.

The use of newly available technologies will be crucial to the progress of research toward development of an effective malaria vaccine. Analysis of serum reactivity profiles using microarray technology offers an opportunity to assess antibody responses to malarial antigens in a high-throughput manner, allowing for the identification of multiple protective antigens in a more efficient process. Although the investigation conducted by Gray et al. (11) showed results for a limited number of malarial antigens, it provides both the laboratory and statistical methodology for assessing antibody response profiles more broadly. In conclusion, while microarray data should be analyzed and interpreted with caution, its use will facilitate the identification of antigens associated with protection.

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References


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